EFFECT OF DIFFERENT ADDITIVES ON THE PRODUCTION OF BACTERIAL CELLULOSE FROM PINEAPPLE WASTE

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ABSTRACT

The pineapple peel wastes contain high concentration of biodegradable organic material such as carbohydrate that can be utilized for the production of organic acid. With the goal of being economically competitive and overcome the problem of disposal of the waste, pineapple waste can be potentially used to enhance the production of bacterial cellulose. Bacterial cellulose is known as polysaccharide and usually it been used traditionally in food industry and the latest it is used as a material for medical application. In this research, bacterial cellulose was produced by *Acetobacter xylinum* from pineapple waste as a fermentation medium with three different types of additives that is sodium alginate, microcrystalline cellulose and carboxymethylcellulose. Hence, the purpose of this experiment is to investigate the effect of three different additives on the production of bacterial cellulose by *Acetobacter xylinum*. Generally, there are three major parts in completing the research on production of bacterial cellulose by *Acetobacter xylinum*. The first part is preparation of inoculum by *Acetobacter xylinum* strain. The inoculum of *Acetobacter xylinum* is mother culture where later on will be used for the next fermentation process for the synthesis of bacterial cellulose. In the second part, the experiment was proceed for bacterial cellulose synthesis. Firstly, the inoculum of *Acetobacter xylinum* was prepared in the HS-medium. After obtaining the optimum yield of bacterial celullose production with different parameter of pH medium, three different additives were used at various concentrations to enhanced bacterial cellulose production. All the medium fermentation was incubated 30°C for 3 days. The optimum pH value was determined by the weight of bacterial cellulose, where the medium fermentations were incubated for three days. The production bacterial cellulose was relatively high with similar properties to that produced in HS medium. Wet weight of bacterial cellulose production by *Acetobacter xylinum* without microcrystalline cellulose was 0.1097g, while bacterial cellulose production was the highest, 0.589 g, at 0.2% MCC. Besides that, the results show that these samples consist of ultrafine fibrils, which form the reticulated structure. The additions of additives into the medium does not effect the structure of the cellulose. These results

suggest that it is possible to produce bacterial cellulose from low-cost resources in order to increase its production to a larger scale.

KESAN ADITIF YANG BERLAINAN TERHADAP PENGHASILAN BAKTERIA SELULOSA DARIPADA SISA NANAS

ABSTRAK

Sisa nanas mengandungi kepekatan tinggi bahan organik terbiodegradasi seperti karbohidrat yang boleh digunakan untuk penghasilan asid organik. Berlandaskan matlamat untuk menjadi ekonomi yang lebih berdaya saing selain mengatasi masalah pelupusan sisa-sisa nanas yang berpotensi digunakan untuk meningkatkan pengeluaran selulosa bakteria. Selulosa bakteria dikenali sebagai polisakarida dan biasanya telah digunakan secara tradisional dalam industri makanan dan yang terbaru ianya digunakan sebagai bahan aplikasi perubatan. Dalam kajian ini, selulosa bakteria telah dihasilkan oleh *Acetobacter xylinum* dengan menggunakan sisa nanas sebagai medium penapaian dengan tiga jenis aditif iaitu alginat natrium, microcrystalline cellulose dan carboxymethycellulose. Justeru itu, tujuan eksperimen ini adalah untuk mengkaji kesan tiga bahan aditif yang berbeza kepada penghasilan selulosa bakteria oleh *Acetobacter xylinum*. Secara umumnya, terdapat tiga bahagian utama dalam menyempurnakan penyelidikan mengenai penghasilan selulosa bakteria oleh *Acetobacter xylinum*. Bahagian pertama adalah penyediaan inokulum *Acetobacter xylinum*. Inokulum *Acetobacter xylinum* adalah sumber inokulum yang mana kemudiannya akan digunakan untuk proses penapaian dan seterusnya untuk sintesis bakteria selulosa. Dalam bahagian kedua, eksperimen telah diteruskan untuk penghasilan bakteria selulosa. Pertama, inokulum *Acetobacter xylinum* telah disediakan dalam medium jus kelapa. Selepas mendapatkan hasil optimum pengeluaran bakteria selullose dengan beberpa nilai parameter media yang berbeza, kemudian tiga aditif yang berbeza telah digunakan pada kepekatan yang berbeza untuk mempertingkatkan pengeluaran bakteria selulosa. Semua media penapaian telah dieram 30 °C selama 3 hari. Nilai pH optimum ditentukan oleh berat daripada bakteria selulosa, dimana media penapaian telah dieram selama tiga hari. Penghasilan bakteria selulosa adalah agak tinggi dengan mempunyai ciri-ciri yang sama yang dihasilkan dalam media-HS. Berat basah penghasilan selulosa bakteria oleh *Acetobacter xylinum* tanpa selulosa adalah 0.1097g, manakala penghasilan selulosa bakteria adalah yang tertinggi, 0,589 g, pada kepekatan selulosa 0.2% w/w. Selain itu, keputusan menunjukkan bahawa sampel ini terdiri daripada gentian yang

amat halus, yang membentuk struktur retikulasi. Penambahan aditif ke dalam medium tidak mempengaruhi struktur selulosa. Keputusan ini menunjukkan bahawa ia adalah tidak mustahil untuk menghasilkan bakteria selulosa daripada sumber kos rendah dalam usaha untuk meningkatkan pengeluaran kepada skala yang lebih besar

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ATP	adenosine triphosphate
BC	bacterial cellulose
CJ-M	coconut juice medium
CMC	caoboxymethylcellulose
FTIR	Fourier Transform Infrared Spectroscopy
HS-medium	Hestrin and Schramm medium
MCC	microcrystalline cellulose
PW-M	pineapple waste medium
SA	sodium alginate
SEM	scanning electron microscopy
TGA	thermogravimetric analysis

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Environmental pollution by waste generated from economic activities are common problems faced by the industries nowadays. World pineapple trade had shown increasing trend for the past three decades. Malaysia, once ranked as one of the top 3 pineapple producers in the world in the 60's and early 70's, has only a relatively modest industry today. The total area under pineapple in the last 5 years was only around 7,000 - 8,000 ha and 5,000 ha are managed by three prominent estates which grow pineapple for canning. Nowadays, Malaysia was listed number 15 of the world fresh pineapple exporter, while for canned pineapple Malaysia was listed as number 9 (MARDI, 2012). Pineapple canning industry is one of the many food industries producing large quantities of waste such as fresh peels. The disposal of the waste is becoming a major problem to many food processing industries. There is a potential for food processing waste such as pineapple waste to be used for conversion into useful and higher value added products. The pineapple peel wastes contain high concentration of biodegradable organic material such as carbohydrate that can be utilized for the production of organic acid. With the goal of being economically competitive and overcome the problem of disposal of the waste, pineapple waste can be potentially used to enhance the production of bacterial cellulose.

Bacterial cellulose is an organic compound and is a form of cellulose that is produced by bacteria. The bacterial cellulose and plant cellulose have same chemical structure but different in physical and chemical properties. Bacterial cellulose is mainly a protective coating while plant cellulose only plays a structural role (Bielecki *et al*, 2000). Bacterial cellulose is synthesized by bacteria belonging to the genera *Acetobacter*, *Rhizobium*, *Agrobacterium*, and *Sarcina* (Jonas and Farah, 1998). However, the only species that can produce enough cellulose for commercial interest is *Acetobacter* species, *Acetobacter xylinum* is able to produces cellulose in high quantity. There are many features of the bacterial cellulose from the methods of the production and one of the most important features of bacterial cellulose its chemical purity. The bacterial cellulose produced by genera *Acetobacter* differs from plant cellulose is its crystallinity and purity (Pourramezan *et al*, 2009).

Bacterial cellulose is known as polysaccharide and usually it been used traditionally in food industry and the latest it is used as a material for medical application (Grande *et al.* 2005). Besides that, bacterial cellulose has application in paper, textile and food industries and also as a biomaterial in cosmetic and medicine (Ring *et al*., 1986). In medical field, several application of bacterial cellulose have been reported such as an artificial skin for human with extensive burns, artificial blood vessel for microsurgery and wound dressing (Czaja *et al.* 2005). While in food industries, bacterial cellulose produced by *Acetobacter xylinum* at the air-liquid interface of coconut water is popularly known as nata-de-coco which use in desserts, fruit cocktails and friut jellies (Jagannath *et al.*, 2008).

In the fermentation process, bacterial cellulose production depends heavily on several factors such as the growth medium and the formation of byproducts. Bacteria are most efficient when supplied with an abundant carbon source and minimal nitrogen source (Ramana *et al*, 2000). Carbon sources played important role for cell growth and bacterial cellulose production as their growth medium, and at the same time cost for bacterial cellulose production must be considered as a main objective. This work aimed to optimize the bacterial cellulose production by using pineapple waste as a substrate with the addition of additives. In this research, three different additives are used to study their effect on bacterial cellulose production.

Recently, there are a few reports stating that addition of water-soluble polymers can increase relative viscosity, hinder coagulation to transfer nutrients and oxygen into bacterial cells, and promote bacterial productivity into the medium (Zhou *et al*., 2007). Addition of different chemicals to fermentation medium in bacterial cellulose production was found to enhance bacterial cellulose production both static and submerged cultivation (Cheng *et al*., 2009).

1.2 PROBLEM STATEMENT

In Malaysia, there are a lot of organic wastes from different stages of agroindustrial productions that, in many cases, cannot be marketed due to their poor quality. However, they are rich in sugars such as glucose, fructose and sucrose, as well as nitrogen and vitamins that are useful for cellulose biosynthesis (Castro *et al.*, 2010). Pineapple peel is the principal solid waste product of the juice processing industry. If these waste discharges to the environment are left untreated they could cause a serious environmental pollution. Then, the industry have to provide proper treatment regarding waste disposal and unfortunately illegal disposal may remain a positive expected present value decision if the penalties are small relative to proper treatment costs (Muoghalu *et al.*, 1990). Rather than allocate lot of money in waste disposal, why not the waste itself is used to generate side income to the company. The used of pineapple residue as raw material in bacterial cellulose production can prevent from the environmental issues.

Meanwhile at the same time, the increasing demand of industrialization for cellulose has imposed extreme negative pressure on the delicate ecoligical balance (Cheng *et al.*, 2009) and one approach to reduce the demand from plants is the production of cellulose using a microbial system (Lynd *et al.*, 2002). However if waste can be transformed into valuable products, this would optimize the profits and competitiveness of the industry. So, the pineapple waste produced from the pineapple canning industries can be used as a substrate for bacterial cellulose production.

In past few years, many researchs has been perform to develop culture media based on other sources of sugars like fruits and vegetables in order to decrease the costs of bacterial cellulose production (Castro *et al.*, 2010). Usage of pineapple residue as substrate in bacterial cellulose production not only optimizing the production of bacteria cellulose, but also lowering the cost of bacterial cellulose production. In addition, the used of pineapple waste as raw material in fermentation medium in bacterial cellulose production also can prevent environmental pollution caused by agroindustrial waste.

1.3 OBJECTIVES OF RESEARCH

i) To investigate the effect of three different additives on the production of bacterial cellulose by *Acetobacter xylinum*.

ii) To optimize the production of bacterial cellulose from pineapple waste by manipulating the pH value of the medium and the concentration of the additives.

1.4 SCOPES OF RESEARCH

i) To produce bacterial cellulose by *Acetobacter xylinum* from pineapple waste as a fermentation medium with three different types of additives which are sodium alginate, microcrystalline cellulose and carboxymethylcellulose.

ii) To examine the relationship between the bacterial cellulose yield and the pH of the fermentation media.

iii) To analyse the production of bacterial cellulose by using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM).

1.5 RATIONAL OF SIGNIFICANT STUDY

i) Reduce the environmental pollution by reuse pineapple waste produced from food and beverage industries in order to optimize the production of bacterial cellulose.

ii) Low cost of baecterial cellulose production.

iii) To obtain high bacterial cellulose yield from the pineapple waste.

CHAPTER 2

LITERATURE REVIEW

2.1 BACTERIAL CELLULOSE

Bacterial cellulose is the basic material of all plant substances and the most abundant polysaccharide on earth. Bacterial cellulose belongs to specific products of primary metabolism (Bielecki *et al.*, 2000). Bacterial cellulose can be extracellularly synthesized into nano-sized fibrils by the bacteria *Acetobactor xylinum*, in presence of glucose in the fermentation medium. One of the characteristics of bacterial cellulose is plant cellulose and bacterial cellulose have the same chemical structure. Recent investigation reveals that bacterial cellulose is chemically identical to plant cellulose (Bielecki *et al.*, 2000). Cellulose can be synthesized by plants, some animal and a large member of microorganisms by the bacteria *Acetobacter xylinum* (Castro *et al.*, 2010).

However, bacterial cellulose possesses particular physicochemical properties different from plant cellulose. There are several aspects that differentiate bacterial cellulose with plant cellulose, which make bacterial cellulose has unique characteristic compared to plant cellulose, including high purity, high crystallinity, high mechanical strength, high water holding capacity, good biocompatibility, and high porosity (Grande *et al*., 2009). Cellulose is the main part in the cell wall and act as protective and coating, whereas plant cellulose (PC) plays a structural role in plant (Bielecki *et al*., 2000). There are no lignin, hemicellulose or other natural components in bacterial cellulose. The unique characteristics make bacterial cellulose an interesting raw material for applications and become potentially important industrial and biomedical material. Tsuchida & Yoshinaga, 1997, suggested that bacterial cellulose is expected to be used for many industrial applications as a high-strength construction material, food additive and a component of biodegradable products and paper. But, one of the bacterial cellulose application problems in industry is its low productivity (Pourramezan *et al*.,2009).

Since the cellulose obtained from the plant is not pure caused it have lignin and hemicellulose, bacterial cellulose used as an alternative instead of plant cellulose in order to produce high purity cellulose and in the same time to reduce the forest depletion as the most cellulose is produced by vascular plant, the increasing demand of industrialization has imposed negative pressure on the plant world (Cheng *et al*.,2009). Besides that, cellulose obtained from wood stock requires the removal of impurities and the process of removal impurities requires lot of the total energy and cause high cost of seperation process. Wider application of this polysaccharide is obviously dependent on the scale of production and its cost (Bielecki *et al*., 2000).

Nowadays, bacterial cellulose used as an alternative instead of plant cellulose in order to produce high purity cellulose and in the same time to reduce the forest depletion (Sherif, 2008). Cellulose is synthesized by bacteria belonging to the genera of *Acetobacter, Rhizobium*, *Agrobacterium*, and *Sarcina* (Retegi *et al*., 2009). The ability to produce high levels of polymer in a large range of carbon and nitrogen sources that caused *Acetobacter xylinum* has applied as a model for the basic and was applied studies on cellulose. To obtain optimum production of bacterial cellulose, it is very critical in selecting the substrates, cultivation conditions, various additives, and finally the bacterial strain (Retegi *et al*., 2009).

2.2 *ACETOBACTER XYLINUM*

Acetobacter xylinum was used as a model bacterium in bacterial cellulose studies by Hestrin *et al*. (1954), who proved that resting and lyophilized *Acetobacter* cells synthesized cellulose in the presence of glucose and oxygen (Bielecki *et al*., 2000). Many strains of *Acetobacter xylinum* are capable of producing cellulose on varying amount and growing on a wide variety of substrates like glucose, sucrose, fructose, invert sugar, ethanol and glycerol (Jagannath *et al*., 2008) and it produced cellulose in the form of extracellular pellicle composed of ribbons. Besides that, oxygen is an important factor for bacterial cellulose production by *Acetobacter xylinum* since it is aerobic microbe (Suwannapinunt *et al*., 2007).

One approach to reduce the demand of bacterial cellulose produced from plants is the production of cellulose using a microbial system (Lynd *et al*., 2002). In this study, cellulose is synthesized by bacteria belonging to the genera *Acetobacter*. This is gram-negative bacterium, strictly aerobic capable of producing extracellulose using glucose, sucrose or others carbon source (Castro *et al*, 2010). *Acetobcater xylinum* is also acetic microbe that growth very well in acid condition from broth medium but *Acetobacter xylinum* still growth because it is a type of acetic microbe and involve in fermentation process to convert glucose to cellulose. Gluconic, acetic or lactic acid is produced by *Acetobacter xylinum* in fermentation process caused the pH decrease from pH 6 to pH 4 in culture medium and at the same time the yield of cellulose decrease in fermentation (Chawla *et al.*, 2008). In alkaline condition, *Acetobacter xylinum* will grow slowly and bacterial cellulose yield will decreasing (Pourramezan *et al.*, 2009). The pH value of the fermentation is one of the most critical parameter since high or low pH value would cause decreasing in cellulose production.

The polymer structure depends on the organism, although the pathway of biosynthesis and mechanism of its regulation are probably common for the majority of bacterial cellulose producing bacteria (Ross *et al.*, 1991). Bielecki *et al.*, 2000, stated the bacterial cellulose is synthesized by several bacterial genera, of which *Acetobacter* strains are best known. Table 2.1 present an overview of bacterial cellulose producers.

Table 2.1 Bacterial cellulose producers

Genus	Cellulose Structure
Acetobacter	extracellular pellicle composed of ribbons
Achromobacter	fibrils
Aerobacter	fibrils
Agrobacterium	short fibrils
Alcaligenes	fibrils
Pseudomonas	no distinct fibrils
Rhizobium	short fibrils
Sarcina	amorphous cellulose
Zoogloea	not well defined

(Source : Jonas and Farah, 1998)

Acetobacter xylinum which is the most efficient producer of cellulose, has been recently reclassified and included within the novel genus *Gluconacetobacter xylunis* (Bielecki *et al.*, 2000).

2.3 PINEAPPLE WASTE AS A SUBSTRATE

In recent years, in order to produce bacterial cellulose at lower cost, there has been many reports to develop method or culture media. One of the advantages of bacterial cellulose is it can be produced from various carbon and nitrogen sources. Various carbon sources including D-glucose, sucrose, fructose, D-galactose, lactose, mannitol and ethanol while for various nitrogen sources are ammonium sulphate, ammonium nitrate, riboflavin, glycine, peptone, sodium nitrate and methionine (Panesar *et al*., 2009).

An alternative method for bacterial cellulose production is using fernentation medium from organic waste. In Malaysia, there are a lot of organic waste from different stages and also type of agroindustrial productions that cannot be marketed due to their poor quality. However, they are rich in sugars such as glucose, fructose, and sucrose as well as nitrogen and vitamins that are essential for fermentation medium in cellulose biosynthesis (Castro *et al*., 2010). Using the pineapple residue and fruit waste such as fruit peel is one of the alternatives that can overcome this problem. The cost of collecting the pineapple rind waste is much lower than buying the pure glucose medium for the cellulose production and these wastes can caused environmental pollution problems if it not is utilized.

Table 2.2 The characteristics of solid pineapple waste reported by different authors.

Composition (%)	Bardiya et al. (1996)	Viswanath (1992)	Chandapillai and Selvarajah (1978)
Moisture	92.80	87.69	89.70
Total solid	7.80	12.31	10.30
Ash	10.60	6.20	3.90
Organic carbon	51.85	38.9	-
Nitrogen free extract	-	-	75.10
Total carbohydrates	35.00	-	-
Ether extract	-	-	0.20
Cellulose	19.80	-	-
Crude fibre	-	-	14.70
Hemicelluloses	11.70	-	-
Phosphorus		0.08	0.10
Total soluble	30.00	-	-
Total nitrogen	0.95	0.90	-

Krueger et al. (1992) have been reported that major constituents of fresh pineapple waste juice are glucose, fructose, sucrose, citric acid, malic acid and mineral potassium.

In Malaysia, the pineapple industry is the oldest agro-based export-oriented industry dating back to 1888. Though relatively small compared to palm oil and rubber, the industry also plays important role in the country's socio-economic development of Malaysia, particularly in Johore. The three registered canneries situated in Johore currently produce all the Malaysian canned pineapple (KPUM, 1990). Malaysian Cannery of Malaysia Sdn. Bhd is a location to obtain the pineapple residue. The canning factory is the first place for the fresh pineapple fruits to be submitted. Then they will be peeled, core removed, sliced, sorted and canned. All the peeled skin, unwanted fruits and the core will be sent to the crush machine for crushing. After crushing, the solid waste will be sent to cattle feeding and in the same